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Chemical Interaction: Enhancement and Inhibition of Clastogenicity

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Most environmental exposures involve concurrent or sequential exposure to multiple chemicals in air, water, and food. Interactive effects in carcinogenesis have been described for certain combinations of agents. They are described in terms of enhancement or inhibition of carcinogenesis. Enhancement effects have been documented for cigarette smoking in combination with exposure to asbestos, radon, alcohol, or other exposures. A variety of inhibitors of carcinogenesis have also been described. They are classified into agents preventing formation of carcinogens; blocking agents; and suppressing agents. Assessment of risk from exposure to multiple agents can be derived either from epidemiological studies in relation to actual exposure or from laboratory studies after controlled exposure to different agents. Prediction of how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions. Compounds may interact chemically, yielding new toxic components or causing a change in the biological availability of the existing components or metabolites. In humans, great individual variability in response is to be expected because of genetic heterogeneity or acquired host susceptibility factors. Interaction is thus a key component in the risk assessment process. In this paper, the definition of interaction and the theoretical basis for different types of interaction in cancer causation are reviewed. Epidemiological and experimental studies showing interactive effects of two chemical carcinogens are also presented.

Introduction

In considering the health risk of exposed populations, it must be borne in mind that people are not exposed to only one potentially hazardous agent. Interactive effects in induction of cancer have been documented from exposure to multiple agents (*1,2*). The term "interaction" describes the way in which the joint effect of two or more agents differs from the simple additive effects. The effects are further classified according to their enhancement or inhibition of carcinogenesis. Such effects constitute an important consideration in assessment of the risk.

Knowledge about interaction in human carcinogenesis is based entirely on epidemiologic studies of effects from cigarette smoking, which is a complete mixture, in conjunction with exposure to other agents. An extensive review of this topic is provided by Saracci (*3*). The report shows enhancement of lung cancer in cigarette smokers by asbestos. Both agents are individually associated with increased risk for development of lung cancer, while combined exposure results in an enhanced risk. Although chronic exposure to asbestos slightly increases the risk of bronchogenic carcinoma in nonsmokers, asbestos workers who smoke are at an 8-fold greater risk than nonexposed smokers and at a 92-fold greater risk than nonsmoking asbestos workers (*4,5*). Enhancement of cancer of the upper alimentary tract was observed in cigarette smokers who consume excessive alcohol (*1*). High incidence of liver cancer was seen in populations with endemic hepatitis and with simultaneous exposure to mycotoxins (*1*). The interaction between alcohol consumption and tobacco smoking has been studied for several sites by Esteve and Tuyns (*6*). They found that the two agents may interact in different ways, depending on the site.

Obviously, the complex mixture of greatest importance in these interactions is cigarette smoke. Another complex mixture that may contribute significantly to the cause of cancer is pyrolyzed products in cooked food (*7*).

Interactions observed in epidemiologic studies are also substantiated in experimental investigations using tobacco smoke condensate, coal combustion fumes, automobile exhausts, and used engine oil (*1*). Enhancement of lung cancer risk from exposure to arsenic and cigarette smoke in smelter workers is supported by the observation of increased chromosomal damage (*8,9*).

Because it is not feasible to test all substances through epidemiological and long-term animal studies, *in vivo* and *in vitro* short-term tests for DNA damage, gene mutation, aneuploidy, chromosomal damage, and cell transformation are viable alternative procedures. The main advantage of such short-term tests is that they are rapid and inexpensive by comparison with other studies (*10*). Data from these tests can be used alone or in groups, as in the genetic activity profile (GAP) database (*11,12*), which provides a computer-generated graphic representation of genetic bioassay data (*13*).

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My laboratory has reported the induction of chromosome damage among traffic policemen in Cairo (*14*). The frequencies of chromosomal aberrations and sister chromatid exchanges are significantly higher among the traffic policemen than in the control group. The increase in chromosomal damage among the traffic policemen is enhanced further by smoking. Another study was conducted to evaluate the cytogenetic effects in male workers exposed to mercury fulminate (*15*). The frequencies of chromosomal aberrations and micronucleated lymphocytes are significantly higher in the exposed group compared to the control group. Smokers in the exposed group showed the highest frequency of chromosomal damage.

In addition to using cells from exposed humans, mice were used in our interaction studies. Praziquantel (PQ) is a commonly used drug to treat patients with schistosomiasis. We have conducted a cytogenetic-urine metabolite study in mice to determine the *in vivo* clastogenic and coclastogenic potential of PQ with a ubiquitous environmental contaminant, benzene. Our study showed that PQ is not clastogenic but can enhance the clastogenic activity of benzene *in vivo* by shifting the metabolic pathway of benzene toward formation of muconaldehyde, which may be responsible for the enhancement effect (*16*). In another study, similar enhancement interaction was observed between benzene and cremophore (*17*). Cremophore El (CR), a frequently used solubilizer and emulsifier in the pharmaceutical and cosmetic industry, is made up of ethylene oxide and caster oil.

Using cells in culture, Hermann (*18*) noticed that several nonmutagenic hydrocarbons with 2- and 3-unsubstituted rings enhanced the mutagenicity of benzo[*a*]pyrene (BaP). Combined treatment of Chinese hamster cells with an extract from automobile exhaust and a mutagen yielded higher mutant frequency than expected [the sum of their individual effects (*19*)]. Dubins and La Velle (*20*) examined the ability of nickel to act as a comutagen with simple alkylating agents in bacterial system. Nickel chloride potentiated the mutagenicity of methyl methanesulfonate in polymerase-proficient strains. Similar results have also been reported for the comutagenic actions of arsenite (*21*), chromate (*22*), and cadmium (*23*) using bacterial assays. Other metal salts such as $CuCl_2$, $MnCl_2$ and $NaMoO_4$ were found to enhance UV mutagenesis in *E. coli* WP2, which has wild-type DNA repair capability (*24*).

Cytoxan interacts with either benzamide, 3-aminobenzamide, or theophylline to induce sister chromatid exchanges (SCEs). The SCE frequencies in the presence of the poly(ADP-ribose)polymerase inhibitors are significantly higher than those in the absence of inhibitor over the cytoxan control. In the same study, the inhibitors in combination with melphalan, thiotepa, or cytoxan act synergistically in causing cell division delay (*25*). Reiss et al. (*26*) observed that simultaneous exposure of adult, male rat liver (ARL-18) epithelial cells to chrysolite asbestos and BaP is more mutagenic than an additive effect of these substances. The clastogenicity of *cis*-diaminedichloroplatinum (II) and 8-methoxypsoralen plus ultraviolet light (UVA) are enhanced by post-treatment with sodium arsenite in Chinese hamster ovary (CHO) cells and in human skin fibroblasts (*27*).

On the other hand, Beckman and Nordenson (*28*) studied the rates of chromosomal aberrations and sister chromatid exchanges in human lymphocytes exposed to combinations of arsenic, lead, and sulfur dioxide, which are the major toxic emissions from copper smelters. No synergistic effects were found. Selenium in combination with the three other agents showed antagonistic effect.

Inhibition of Carcinogenesis

A variety of inhibitors of carcinogenesis have been described. They are classified into agents preventing formation of carcinogens, blocking agents, and suppressing agents (*1*). Selenium, for instance, has been found to have a protective effect against chromosomal damage induced by arsenic (*29*). It showed an antagonistic (protective) effect against sodium arsenite, lead acetate and sodium sulfite (*28*).

A number of dietary components have been identified as inhibitors of mutagenesis induced by various chemical mutagens. These include vitamins, trace metals, fatty acids, protease inhibitors, polyphenols, porphyrins, sulfhydryl compounds, essence of vanilla and cinnamon oil, and several other agents (*30*). Among vitamins, retinoids, tocophenol, ascorbic acid, and riboflavin were shown to inhibit mutagenicity of a number of chemical mutagens (Table 1).

In one of our studies (*31*), we demonstrated that the mutagenic and potentially carcinogenic activity of benzene can be blocked by a free-radical scavenger, dimethyl sulfoxide (DMSO). Therefore, the potential use of free-radical scavenger to protect workers exposed to benzene may be considered.

Mechanisms of Interaction

Predicting how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions. The interaction may occur

Table 1. Examples of dietary inhibitors of mutagenesis (*30*).

Dietary agent	Test system	Inhibit the mutagenic activity of
Vitamin A	*S. typhimurium*	Cyclophosphamide (CP), protein pyrolysates, aflatoxin B_1, benzo[*a*]pyrene (BaP)
	V79 CHO cells	CP, aflatoxin B_1
Vitamin E	Fibroblast of golden hamster	Methyl mercury
	Drosophila	Radiation
	CHO cells	BaP
Ascorbic acid	*S. typhimurium*	Dimethylnitrosamine
	Cells in exposed workers	Polyaromatic hydrocarbons, benzene
Selenium	Bacteria, human cells	Nitrosamines, BaP
Cobalt	*E. coli*	UV, γ rays

CHO, Chinese hamster ovary cells.

during any stage of the toxicological process: absorption, distribution, metabolism, and excretion. Compounds may also interact chemically, yielding new toxic components or causing a change in the biological availability of the existing components or metabolites. In humans, great individual variability in response is to be expected because of genetic heterogeneity or acquired host susceptibility factors.

Hermann (*18*) stated that the higher the number of cycles of the polycyclic aromatic hydrocarbons (PAH), the lower the amount of PAH necessary to enhance and/or inhibit the mutagenicity of BaP. Metabolic activation of BaP was evidently involved in synergistic phenomenon because no effect was observed on BaP-4,5 oxide, a direct-acting metabolite of BaP. In the presence of exogenous activation, enhancement may also be due to competition for deactivating enzymes, as suggested by Ashby and Styles (*32*). After activation of mutagen, the expression of genotoxicity, such as chromosomal aberrations, can be modified by DNA repair processes (*33*).

Reiss et al. (*26*) discussed several possible means by which asbestos fibers could cause enhancement of BaP mutagenesis. The fibers may adsorb BaP and/or affect cellular membrane structure. These activities would facilitate transport of BaP across the plasma membrane. Asbestos fibers may also increase the binding of BaP to isolated microsomes. This property of asbestos could increase the availability of the carcinogen for metabolic activation. Nickel could affect DNA repair by decreasing the fidelity of DNA synthesis via some action on DNA polymerases (*20*). Moreover, Rossman and Molina (*24*) suggest that the comutagenic effects of metals might occur either via metal induced infidelity of repair replication or (in case of $CuCl_2$) via metal-induced depurination. Lee et al. (*27*) observed that the cogenotoxic activity of sodium arsenite is confined to interaction with S-dependent agents. Such activities may be due to inhibition of repair of the induced lesions, induction of error prone repair or interference with DNA replication.

The mechanisms of desmutagenesis have been proposed to involve chemical inactivation of mutagens, enzymatic inactivation of mutagens, inhibition of metabolic activation of promutagens, and inactivation of activated mutagens, including scavenging processes (*34*).

Risk Assessment of Interaction

Interaction between different agents in a mixture can produce effects either less than (antagonistic) or greater than (synergistic) the sum of the effects of individual components. This will lead to underestimation or overestimation of the actual risks (*1,28*). Therefore, in estimation of risk, potential interaction among different agents should be considered (*2*).

The first step of a risk assessment process is probably the documentation of mutagen burden, which is referred to as the average amount of any given mutagenic chemical that was unavoidably ingested, absorbed, or inhaled from the environment and/or produced and carried endogenously within the body (*35*). The net total burden would take into account the amount of antimutagens that also might enter the body. Unfortunately, at the present time there is no practical way to measure such net or total burden. The problem is complicated further by the fact that mutagen doses vary at different stages along the pathway from exposure to biological response. However, it is possible in the future that surveys of the mutagenicities of various body fluids, coupled with monitoring, could provide some useful insight into the magnitudes of these quantities (*36*).

A mathematical approach to understanding interaction was applied to the interaction between two or more toxic agents (*37*). The results indicate the existence of a strong synergistic interaction between ethyl methanesulfonate and ultraviolet light for cell killing in the diploid yeast. Application of such mathematical models may help in risk assessment of combined exposure.

Information generated by using mathematical models may complement data generated by epidemiological studies and from laboratory studies after exposure to different agents. Prediction of how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions.

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